PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Docket No: Q77153
Kazuhiro AIKAWA	
Appln. No.: 10/670,004	Group Art Unit: 1615
Confirmation No.: 6236	Examiner: Gollamudi S. KISHORE
Filed: September 25, 2003	

For: LIPOSOME CONTAINING REMEDY FOR VASCULAR DISEASE

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Kazuhiro AIKAWA, hereby declare and state as follows:

I am a citizen of Japan.

I received a Master's Degree of Science from Gakushuin University in March 1982.

In April 1982, I accepted a position as a researcher with Fuji Photo Film Co., Ltd. (now FUJIFILM Corporation). I am at present doing research work on contrast agents at FUJIFILM's Advanced Research Laboratories.

I am familiar with the Office Action mailed September 17, 2007 on the present application.

I am aware that claims 1 and 4-6 are rejected under 35 U.S.C. § 102(b) as being anticipated by EP 0 583 665.

I am aware that claims 1 and 4-6 are rejected under 35 U.S.C. § 103(a) as being unpatentable (obvious) over EP 0 583 665 in view of Aikawa (7,101,532) or Kitaguchi (7,008,614) or Schmidt (6,077,529) individually or in combination.

I am also aware that claims 1 and 4-6 are rejected under 35 U.S.C. § 103(a) as being unpatentable (obvious) over Aikawa (5,387,600) in view of Aikawa (7,101,532) or Kitaguchi (7,008,614) or Schmidt (6,077,529) individually or in combination.

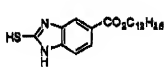
With regard to the §103 obviousness rejections, I have conducted comparative experiments to demonstrate that a benzimidazole compound incorporated as a membrane component in the liposome achieves superior (i.e., higher) uptake by macrophages in comparison to a benzimidazole compound that is separately added to macrophages together with liposomes in which the benzimidazole compound has not been incorporated in the liposome membrane. The comparative experiments are described below:

Comparative Experiments

ICR male mice (6-week old, Charles River Japan) were acclimatized for 1 week. Under anesthesia with ether, almost whole blood was collected from the carotid artery of each mouse. PBS (GIBCO, NO. 1254-087, 5 ml) was intraperitoneally injected and the abdomen was sufficiently rubbed, and then the PBS was recovered. The recovered PBS was placed in a centrifuge tube and subjected to centrifugation at 3,000 r.p.m. for 5 minutes. The supernatant was discarded and then the precipitate was added with E-MEM medium (GIBCO, No. 1263-002) to prepare a macrophage suspension (1×10^6 macrophages/ml). The macrophage suspension was added to each well of a 12-well plate (Corning, No. 012, 1 ml/well) and the cells were allowed to stand for 2 hours under 5% CO_2 at 37°C. The cells were washed and the medium was replaced with fresh E-MEM, and then the cells were incubated for 24 hours under 5% CO_2 at 37°C.

The resulting macrophages in the wells were mixed with a benzimidazole compound and liposomes according to each of the following procedures (Sample 1 as a reference) and then incubated for 24 hours under 5% CO_2 at 37°C. In each of the samples, the benzimidazole compound was adjusted to a concentration of 5μM as a final concentration in the mixture.

Sample 1: The macrophages mixed with a benzimidazole compound disclosed as Compound 34 in Japanese Patent Unexamined Publication (KOKAI) No. (Hei) 6-48942 shown below (hereinafter referred to "Compound I").

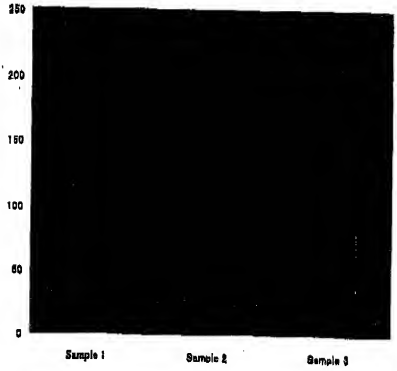


Sample 2: The macrophages first mixed with Compound I and then further added with liposomes prepared by using PC:PS=50:50 (nmol),[1]

Sample 3: The macrophages mixed with liposomes that were prepared by using PC:PS:Compound I=50:50:10 (nmol).

The macrophages after the incubation were washed and added with a detergent to obtain a solution, and then the concentration of Compound I uptaken in the macrophages in each sample was measured using high-performance liquid chromatography (HPLC). The results are shown below. In the figure, the vertical axis indicates the concentration of Compound I uptaken in the macrophages (nmol). As clearly demonstrated by these experimental results, the benzimidazole compound added after incorporation in the liposome (PC:PS:Compound I) gave remarkably higher concentration, i.e., about 10-fold higher concentration, as compared with Sample 2 wherein the benzimidazole compound was added separately with the liposome (PS:PS + Compound I). In fact, there is no difference in the uptake of Compound I into the macrophages of Sample 1 (Compound I without liposome) and Sample 2 (PS:PS + Compound I). In my opinion, the superior results obtained with the liposomes of the present invention would have been unexpected to a person of ordinary skill in the art.

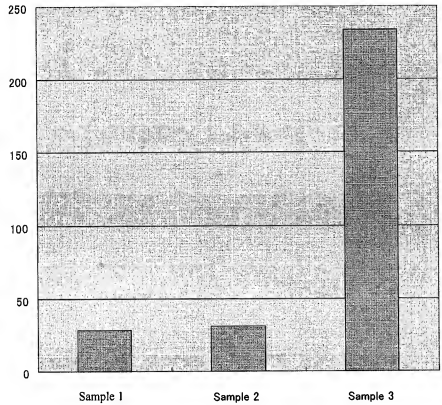
1 "PC" refers to phosphatidylcholine. "PS" refers to phosphatidylserine.



I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: January 15, 2008

Kazuhiro Aikawa
Kazuhiro AIKAWA



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Date: _____

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Kazuhiro AIKAWA